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NCAPG, mediated by miR-378a-3p, regulates cell proliferation, cell cycle progression, and apoptosis of oral squamous cell carcinoma through the GSK-3β/β-catenin signaling

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Exploring the molecular mechanism of oral squamous cell carcinoma (OSCC) pathogenesis is of great significance for its improvement and therapy. Non-structural maintenance of chromatin condensin I complex subunit G (NCAPG) is responsible for chromatin condensation and is associated with the progression of many malignant tumors. This study was aimed to investigate the role of NCAPG on OSCC pathogenesis. NCAPG mRNA expression data in OSCC tissues were obtained from the Gene Expression Omnibus (GEO) database and NCAPG protein expression in OSCC cell lines was determined by western blotting analysis. The results demonstrated that NCAPG expression in OSCC tissues and cells was higher than that of normal control. Following the short interfering RNA (siRNA) knockdown of NCAPG in two OSCC cell lines, we observed that NCAPG depletion notably inhibited OSCC proliferation and cell cycle progression, as well as promoted apoptosis *in vitro*. Besides, silencing of NCAPG specifically inhibited the GSK-3β/β-catenin signaling. Furthermore, we demonstrated that NCAPG was a downstream target of miR-378a-3p. NCAPG silencing counteracted the effect of the miR-378a-3p inhibitor on cell proliferation/cycle induction. Collectively, these findings suggest that NCAPG is crucial in OSCC progression and development, and may serve as a potential therapeutic target for OSCC.

Key words: NCAPG, miR-378a-3p, oral squamous cell carcinoma, GSK-3β/β-catenin

Head and neck squamous cell carcinomas (HNSCCs) are among the most prevalent malignancies globally and have serious public health and economic burdens [1]. Oral squamous cell carcinoma (OSCC) is a type of heterogeneous cancer arising from the mucosal lining of the oral cavity and is the most common type of HNSCC [2]. With regard to epidemiologic trends, the incidence of OSCC is rising in numerous countries over the past decades [3, 4]. Despite great advances that have been made in its diagnosis and treatment, the 5-year survival rate of patients with OSCC remains at an unsatisfactory level [5]. Hence, it is of great significance to gain insights on OSCC pathogenesis to verify novel candidate molecules and develop efficacious therapeutic approaches.

Non-structural maintenance of chromatin condensin I complex subunit G (NCAPG) is encoded by the NY-MEL-3 gene that is located on human chromosome band 4p15.32 [6]. It is a mitosis-related chromosome condensation protein existing in the condensin I complex [7], and is responsible for chromosome condensation and stabilization during meiosis and mitosis [8]. Recent evidence has revealed the carcinogenic role of NCAPG in distinct cancer types, including hepatocellular carcinoma [9, 10], cardia adenocarcinoma [11, 12], prostate cancer [13], and breast cancer [14]. However, the functional role of NCAPG in OSCC is still unrevealed.

Herein, we analyzed RNA expression data from the Gene Expression Omnibus (GEO) database to identify the aberrantly expressed genes in OSCC. It turned out that NCAPG was significantly upregulated in OSCC tissues compared to normal tissues according to the expression data of GSE37991, GSE23558, and GSE30784 profiles. Therefore, we predicted NCAPG as an oncogene in OSCC progres-

sion. We verified this opinion with multiple bio-functional experiments and further explored the underlying molecular mechanism in this study.

Materials and methods

Acquisition of gene expression data. Gene expression data in GSE37991, GSE23558, and GSE30784 profiles were obtained from the GEO database (https://www.ncbi.nlm.nih.gov/gds/) and analyzed using GEO2R tools (http://www.ncbi.nlm.nih.gov/geo/geo2r/). The array data for GSE37991 included 40 OSCC samples and 40 normal oral epithelial samples. The array data for GSE23558 included 27 OSCC samples and 4 normal oral epithelial samples. The array data for GSE30784 included 167 OSCC samples and 45 normal oral epithelial samples.

Cell sources, culture, and transfection. Human oral mucosal epithelial cells (hOMECs), human OSCC cell lines CAL-27 and FaDu were purchased from iCell Bioscience Inc. (Shanghai, China). Human OSCC cell line UPCI-SCC-090 and human embryonic kidney 293T cells were purchased from Zhong Qiao Xin Zhou Biotechnology Co., Ltd. (Shanghai, China). Human OSCC cell line SCC-25 was purchased from Procell Life Science &Technology Co., Ltd. (Wuhan, China).

hOMECs were cultured in a primary epithelial cell culture medium (iCell). CAL-27, SCC-25, and 293T cells were cultured in DMEM (Servicebio Technology Co., Ltd., Wuhan, China) supplemented with 10% FBS (Tianhang Biotechnology Co., Ltd., Hangzhou, China). FaDu and UPCI-SCC-090 cells were cultured in MEM (Solarbio Science & Technology, Co., Ltd., Beijing, China) supplemented with 10% FBS. All cells were maintained in a humidified chamber containing 5% CO_2.

Cells were transfected with small interference RNAs (siRNAs) targeting NCAPG to knock down NCAPG expression using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA). Three candidate NCAPG siRNA (siNCAPG) sequences and one negative control siRNA (siNC) were used. The sequences of the siRNA oligos targeting to NCAPG were as follows: siNCAPG#1: 5'-ACCGCACGATGGATGATAA-3' (forward) and 5'-TTATCATCCATCGTGCGGT-3' (reverse); siNCAPG#2: 5'-GCACCATCAGCAAAGACTT-3' (forward) and 5'-AAGTCTTTGCTGATGGTGC-3' (reverse); siNCAPG#3: 5'-TGCCCAGTGGTTAATGCAT-3' (forward) and 5'-ATGCATTAACCACTGGGCA-3' (reverse); The siNC sequence was 5'-UUCUCCGAACGUGUCACGUTT-3' (forward) and 5'-ACGUGACACGUUCGGAGAATT-3' (reverse). The two most efficient interference sequences were selected for further investigation. Additionally, cells were transfected with miR-378a-3p mimic, miR-378a-3p inhibitor, or their negative controls using Lipofectamine 3000 reagent to overexpress or knockdown miR-378a-3p expression. Their sequences were as follows: NC mimic: 5'-UUCUCCGAACGUGUCACGUTT-3' (forward) and 5'-ACGUGACACGUUCGGAGAATT-3' (reverse); miR-378a-3p mimic: 5'-ACUGGACUUGGAGUCAGAAGGC-3' (forward) and 5'-CUUCUGACUCCAAGUCCAGUUU-3' (reverse); NC inhibitor: 5'-UUGUACUACACAAAAGUACUG-3'; miR-378a-3p inhibitor: 5'-GCCUUCUGACUCCAAGUCCAGU-3'.

Western blotting. Proteins were extracted from cultured cells using RIPA lysis buffer (Solarbio). After centrifugation at 10,000×g for 5 min at 4 °C, proteins were collected and concentration was determined using a BCA protein assay kit (Solarbio). An equal amount of proteins of each sample was separated by SDS-PAGE (Solarbio) and the separated proteins were then transferred onto PVDF membranes (Millipore, Billerica, MA, USA). After blocking in 5% skimmed milk for 1 h, membranes were incubated with NCAPG antibody (A13123; 1:1000; ABclonal Biotechnology Co., Ltd., Wuhan, China), PCNA antibody (A12427; 1:1000; ABclonal), Cyclin D1 antibody (A19038; 1:500; ABclonal), CDK1 antibody (A11420; 1:500; ABclonal), caspase-3 antibody (#14220; 1:1000; Cell Signaling Technology, Danvers, MA, USA), caspase-9 antibody (#9508; 1:1000; Cell Signaling Technology), phospho-GSK3β antibody (AP1088; 1:500; ABclonal), GSK3β antibody (A6164; 1:1000; ABclonal), phospho-β-catenin antibody (DF2989; 1:500; Affinity Biosciences, Cincinnati, OH, USA), β-catenin antibody (AF6266; 1:500; Affinity), and GAPDH antibody (60004-1-Ig; 1:10000; Proteintech Group, Inc., Rosemont, IL, USA) at 4 °C overnight. After washing three times, membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (Solarbio) at room temperature for 1 h. The blots were visualized using enhanced chemiluminescence (ECL) reagent (Solarbio) in a gel imaging system (Liuyi Biotechnology Co., Ltd., Beijing, China). This experiment was repeated three times.

Cell counting kit-8 (CCK-8) assay. Cells (3,000/well) were placed in a 96-well plate in quintuplicate and transfected as experiment design. After transfection for 0, 1, 2, 3, and 4 days; 10 μl of CCK-8 reagent (Beyotime Institute of Biotechnology Co., Ltd., Shanghai, China) was added to each well and further incubated for 2 h. The optical density (OD) at 450 nm wavelength was then detected using a microplate spectrophotometer to reflect the cell viability. This experiment was repeated three times.

EdU assay. Cells were transfected according to the experiment design. After transfection for 2 days, an EdU imaging kit (KeyGen Biotech. Co. Ltd., Nanjing, China) was applied for assessment of cell proliferation following the manufacturer's instructions. The cellular nucleus was stained with Hoechst33342. Staining results were imaged using an inverted phase-contrast microscope. This experiment was repeated three times.

Flow cytometry. For assessment of the cell cycle progression, cells were transfected according to the experiment design. After transfection for 2 days, cells were collected via centrifugation at 1000×g for 5 min and then fixed with 70%

ethanol. Next, cells were stained with 1% propidium iodide (PI, Beyotime) and 10 µl of RNase enzyme (Beyotime) for 30 min in the dark. A flow cytometer was used to detect cell cycle distribution immediately. This experiment was repeated three times.

For assessment of apoptosis, cells were transfected according to the experiment design. After transfection for 2 days, cells were collected via centrifugation at 310×g for 5 min and then resuspended with 500 µl of binding buffer. Cells were then stained with 5 µl of AnnexinV-fluorescein isothiocyanate (FITC, KeyGen) and 5 µl of PI (KeyGen) for 15 min in the dark. A flow cytometer was used to detect cell apoptosis immediately. This experiment was repeated three times.

Dual-luciferase reporter assay. miRDB bioinformatics database (http://mirdb.org/) was used to predict the binding sites of hsa-miR-378a-3p and NCAPG. pmirGLO vectors encoding NCAPG-3'-UTR-Wt and NCAPG-3'-UTR-Mut were constructed. 293T cells were co-transfected with NCAPG-3'-UTR-Wt or NCAPG-3'-UTR-Mut plasmids and miR-378a-3p mimic or NC mimic using Lipofectamine 3000 reagent. Following transfection for 48 h, a luciferase detection kit (KeyGen) was applied to determine the luciferase activities following the kit's protocols. Renilla luciferase activity was normalized to firefly luciferase activity. This experiment was repeated three times.

Statistical analysis. Data are presented as mean ± SD from three independent experiments. Differences were compared using GraphPad 8 software with unpaired Student's t-test or one-way analysis of variance (ANOVA) followed with Tukey's multiple comparisons test. A p-value <0.05 was indicative of statistical significance.

Results

NCAPG is upregulated in OSCC tissues and cell lines. In order to examine the status of NCAPG expression in OSCC tissues, GEO profiles GSE37991, GSE23558, and GSE30784 downloaded from the GEO database were employed, and gene expression data in each profile were analyzed using GEO2R. The results showed that the NCAPG expression was significantly upregulated in tumor samples compared to normal samples (Figures 1A–1C). Furthermore, to examine the status of NCAPG expression in OSCC cells, four OSCC

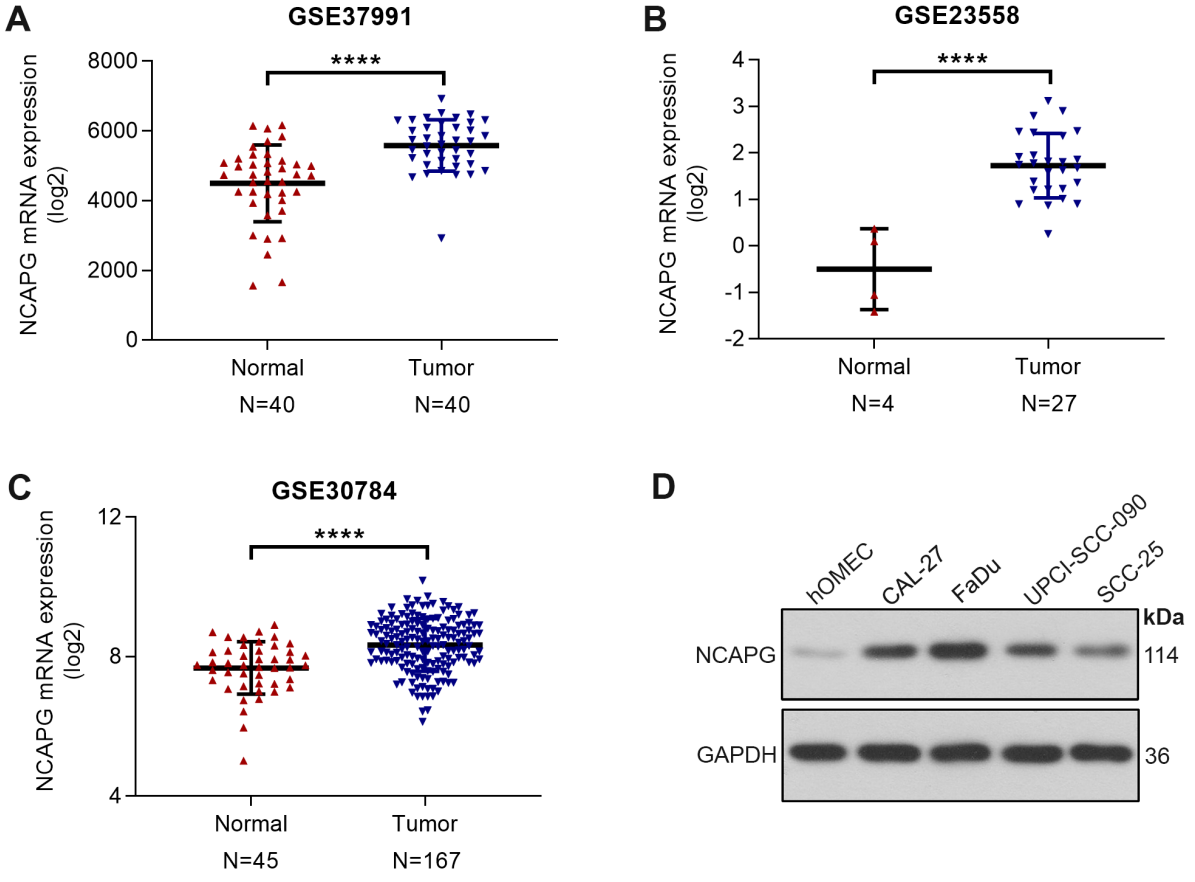

Figure 1. Upregulation of NCAPG in OSCC tissues and cell lines. A–C) Analysis of NCAPG mRNA expression in OSCC datasets from the Gene Expression Omnibus (GEO) database. D) Western blotting analysis of NCAPG expression in one human oral mucosal epithelial cell line (hOMEC) and 4 human OSCC cell lines (CAL-27, FaDu, UPCI-SCC-090, and SCC-25). p-values are calculated by a two-tailed, unpaired t-test. ****p<0.0001; compared with the normal group.

cell lines (CAL-27, FaDu, UPCI-SCC-090, and SCC-25) and one oral mucosal epithelial cell line (hOMEC) were employed. Results of western blotting showed that NCAPG protein levels in all four OSCC cell lines were higher than that of hOMEC (Figure 1D). Two cell lines with the highest NCAPG expression (FaDu and CAL-27) were chosen for functional examinations.

NCAPG expression was suppressed by siRNA transfection in OSCC cells. To assess the role of NCAPG in OSCC progression, we used siRNAs to silence the NCAPG expression to perform the loss-of-function experiments. Three siRNAs were constructed and transfected into FaDu and CAL-27 cells. Western blotting analysis showed that the NCAPG expression was obviously decreased in OSCC cells after siNCAPG transfection (Figure 2). Among the three siRNAs, siNCAPG#1 and siNCAPG#2 showed better knockdown efficiencies. Therefore, siNCAPG#1 and siNCAPG#2 were chosen for further studies.

Knockdown of NCAPG inhibits OSCC cell proliferation. CCK-8 assay and EdU assay were applied to assess the effect of NCAPG knockdown on OSCC cell proliferation. As shown in Figure 3A, the proliferative abilities of cells in two siNCAPG transfection groups were significantly inhibited compared to that of the siNC transfection group at time points of 2, 3, and 4 days in both FaDu and CAL-27 cells. Similarly, the proliferative cells in two siNCAPG groups were markedly reduced compared to the siNC group (Figures 3B, 3C). Moreover, we also detected the expression of a proliferation marker, PCNA, after siRNA transfection. Consistently, we found that its expression was obviously decreased in two siNCAPG groups compared to the siNC group (Figures 3D, 3E) in both FaDu and CAL-27 cells. These results demonstrated the regulatory role of NCAPG in OSCC cell proliferation.

Knockdown of NCAPG induces OSCC cell cycle arrest. Cell cycle distribution in FaDu and CAL-27 cells after transfection with siNC or siNCAPGs were determined by flow cytometry. As shown in Figures 4A and 4B, siNCAPG knockdown significantly increased the proportion of cells in the G1 phase and decreased the proportion of cells in the S phase. Besides, the expression of cell cycle checkpoint markers cyclin D1 and CDK1 were also found downregulated in NCAPG-knockdown OSCC cells (Figures 4C, 4D). These results indicated the regulatory role of NCAPG in OSCC cell cycle progression.

Knockdown of NCAPG accelerates OSCC cell apoptosis. Apoptotic OSCC cells were determined by flow cytometry. As shown in Figures 5A and 5B, siNCAPG knockdown significantly increased the number of apoptotic OSCC cells. Moreover, two apoptosis-related molecules caspase-3 and caspase-9 were examined by western blotting analysis. The results showed that siNCAPG knockdown notably increased the cleaved-caspase-3 and cleaved-caspase-9 levels compared to siNC transfection (Figures 5C, 5D). These results suggested the regulatory role of NCAPG in OSCC cell apoptosis.

Knockdown of NCAPG suppresses the GSK-3β/β-catenin signaling in OSCC cells. To explore the possibly involved signaling pathway in the regulatory role of NCAPG, key molecules in the GSK-3β/β-catenin signaling were detected. The results of western blot analysis suggested that NCAPG knockdown effectively inhibited the phosphorylation of GSK-3β and promoted the phosphorylation of β-catenin (Figures 6A, 6B), indicating that NCAPG may affect the transduction of the GSK-3β/β-catenin pathway.

To investigate whether NCAPG silencing specifically affected the GSK-3β/β-catenin signaling, we further treated the transfected OSCC cells with human rWNT5A. Briefly, after cells were transfected with siNCAPG or siNC for 32 h, rWNT5A (200 ng/ml) was added and then incubated for another 16 h. The results showed that the addition of rWNT5A counteracted the effects of NCAPG silencing on the GSK-3β/β-catenin pathway-related molecules (Figures 6C, 6D), indicating that NCAPG specifically regulated the transduction of the GSK-3β/β-catenin signaling.

miR-378a-3p was a potential upstream regulator of NCAPG. Our previous work has demonstrated that miR-378a-3p acts as a tumor suppressor in OSCC progression. By using the miRDB bioinformatics database, we predicted NCAPG as a target gene of miR-378a-3p. To investigate whether miR-378a-3p affected NCAPG expression in OSCC cells, we treated FaDu and CAL-27 cells with miR-378a-3p mimic or inhibitor. It turned out that NCAPG

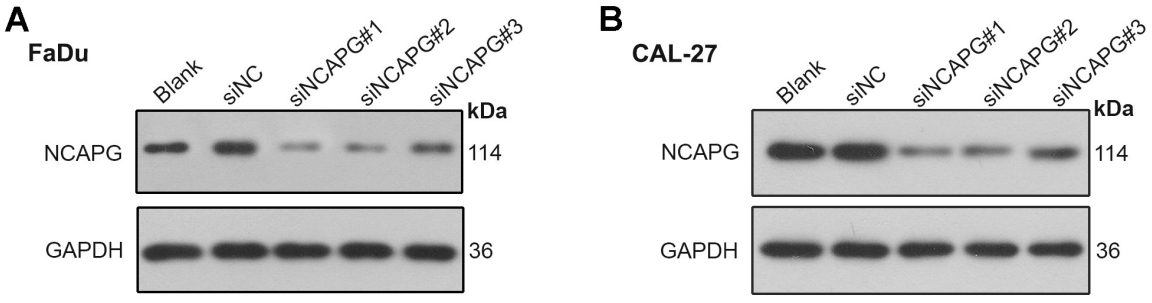

Figure 2. NCAPG expression was suppressed by siRNA transfection in OSCC cells. Effects of siNCAPG transfection on NCAPG expression in two OSCC cell lines FaDu and CAL-27, as detected by western blotting analysis.

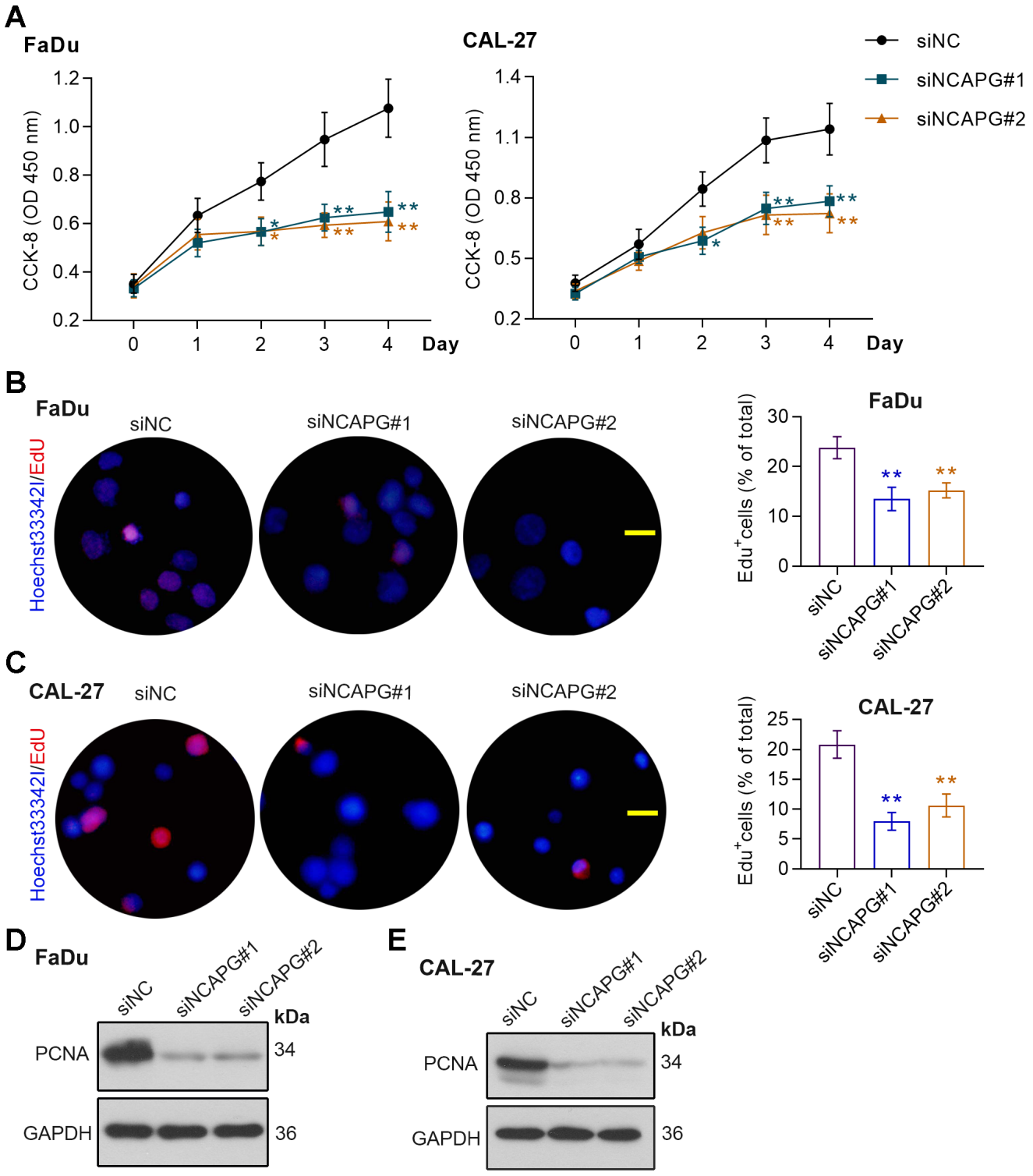

Figure 3. Knockdown of NCAPG inhibited the proliferation of OSCC cells. Effects of NCAPG knockdown on cell proliferation of two OSCC cell lines FaDu and CAL-27 using CCK-8 assays (A) and Edu assays (B, C). Scale bar = 20 μm. Effects of NCAPG knockdown on the expression of a proliferation marker (PCNA) using the western blotting analysis on FaDu (D) and CAL-27 cells (E). P values are calculated by one-way ANOVA. *p<0.05; **p<0.01; compared with the siNC group.

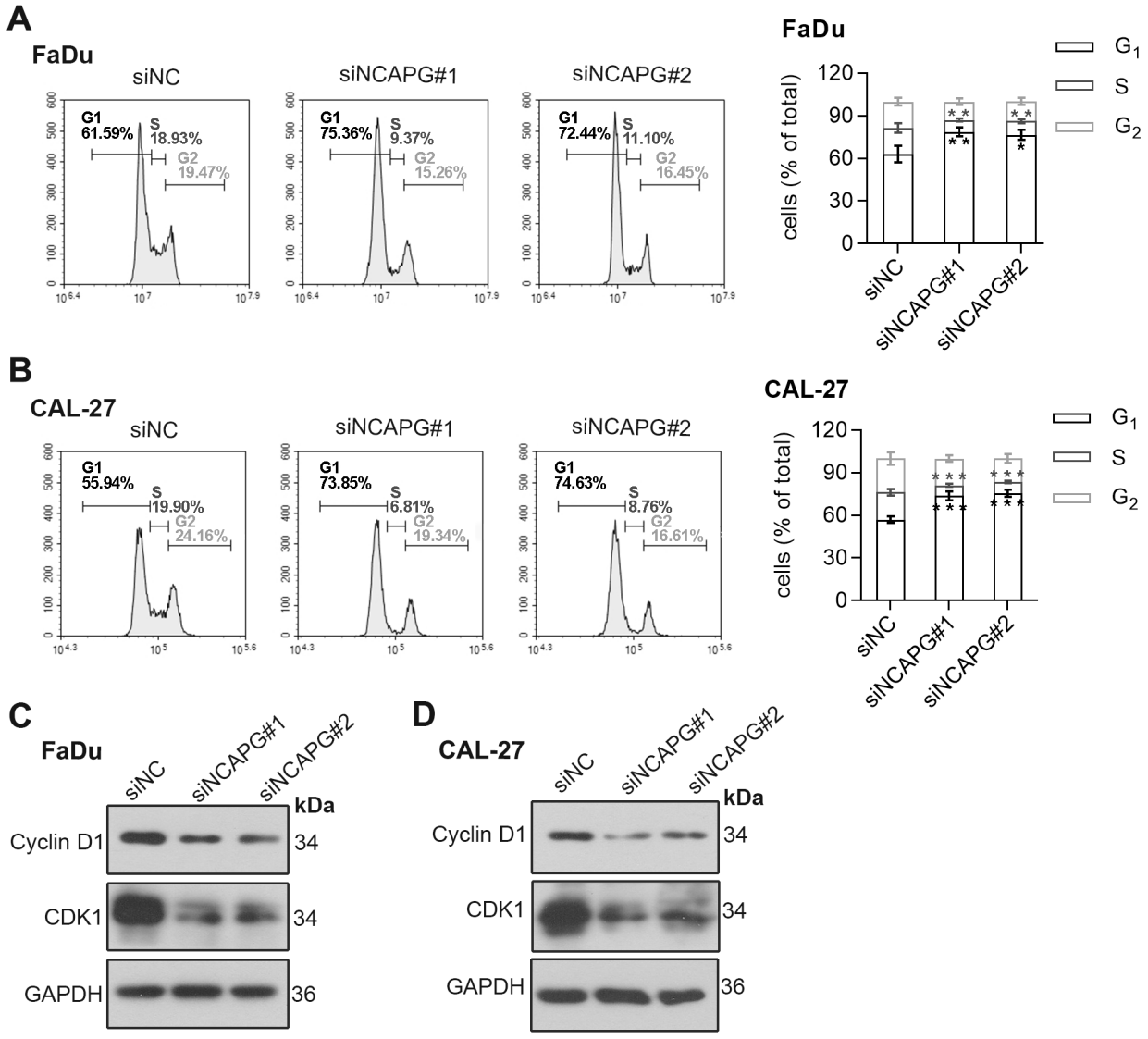

Figure 4. Knockdown of NCAPG induced the cell cycle arrest of OSCC cells. Effects of NCAPG knockdown on the cell cycle distribution of two OSCC cell lines FaDu (A) and CAL-27 (B) using flow cytometry. Effects of NCAPG knockdown on the expression of cell cycle markers (Cyclin D1 and CDK1) using the western blotting analysis on FaDu (C) and CAL-27 cells (D). p-values are calculated by one-way ANOVA. *p<0.05; **p<0.01; ***p<0.001; compared with the siNC group.

protein level was reduced after transfection with miR-378a-3p mimic, whereas elevated after transfection with miR-378a-3p inhibitor (Figures 7A, 7B).

Besides, to verify the direct binding between NCAPG and miR-378a-3p, 293T cells were co-transfected with NCAPG-3'-UTR-Wt or NCAPG-3'-UTR-Mut plasmid and miR-378a-3p mimic or NC mimic, and a dual-luciferase assay was performed subsequently. The results showed that the luciferase activity of NCAPG-3'-UTR-Wt co-transfected with the miR-378a-3p mimic was significantly downregulated compared to that with the NC mimic. Additionally, there was no significant difference between the NCAPG-3'-UTR-Mut co-transfected with miR-378a-3p mimic and NCAPG-3'-UTR-Mut co-transfected with NC mimic (Figure 7C), indicating that miR-378a-3p directly binds to the NCAPG-3'-UTR.

Furthermore, to investigate whether miR-378a-3p exerts its anti-tumor effect by regulating NCAPG, a rescue experiment was designed and performed. As expected, siNCAPG partially abolished the enhancement of miR-378a-3p inhibitor on OSCC cell proliferation and cell cycle progression (Figure 8). These results implicated that miR-378a-3p is a potential upstream regulator of NCAPG in OSCC progression.

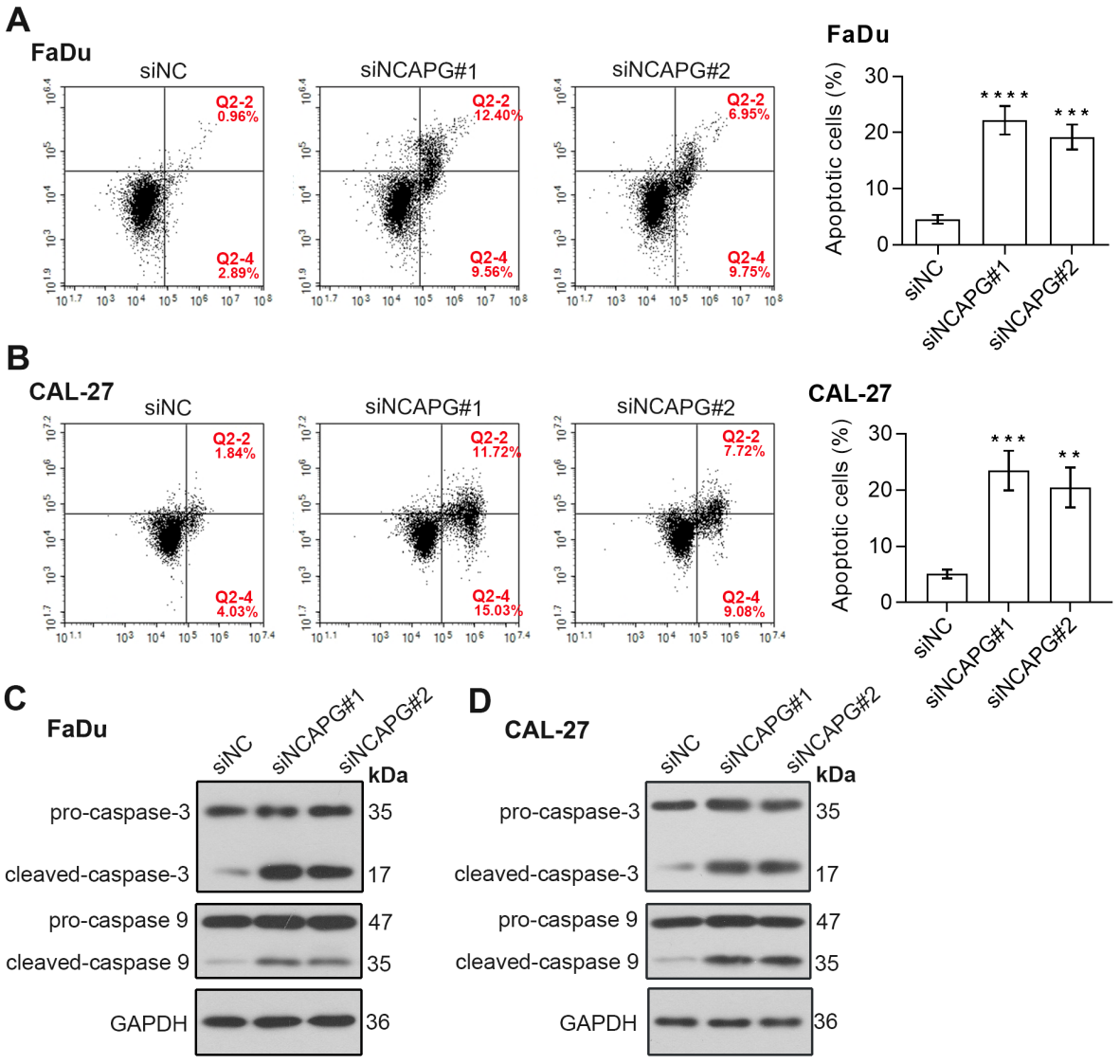

Figure 5. Knockdown of NCAPG accelerated the apoptosis of OSCC cells. A, B) Effects of NCAPG knockdown on the apoptosis of two OSCC cell lines FaDu (A) and CAL-27 (B) using flow cytometry. C) Effects of NCAPG knockdown on the expression of apoptosis markers (caspase-3 and caspase-9) using the western blotting analysis on FaDu (C) and CAL-27 cells (D). p-values are calculated by one-way ANOVA. **p<0.01; ***p<0.001; ****p<0.0001; compared with the siNC group.

Discussion

OSCC is the main subset of HNSCC and has high incidence and mortality [15]. Patients diagnosed with OSCC usually experience a poor quality of life [16]. Although surgery, chemotherapy, biological therapy, and radiotherapy have made continuous progress in OSCC treatment clinically, the survival rate of advanced OSCC patients is still very low because of its poor prognosis and recurrence [17]. Understanding the pathogenesis of OSCC is valuable for the identification of novel biomarkers and the establishment of new treatment modalities [18].

In our effort to identify crucial molecular targets in OSCC progression, we preliminarily found that NCAPG was markedly upregulated in OSCC tissue samples compared to normal oral mucosal or epithelial tissue samples by searching bioinformatics resources. We further validated the NCAPG overexpression in OSCC cell lines. NCAPG is a polypeptide composed of 1,015 amino acids with a relative molecular weight of 114.1 kDa [19]. It has been reported that NCAPG correlates with ATPase activity, tubulin binding and catalytic activity, chromosome segregation, and condensation process, and is closely involved in DNA replication, mismatch repair, cell cycle progression, and cancer-related signaling pathways

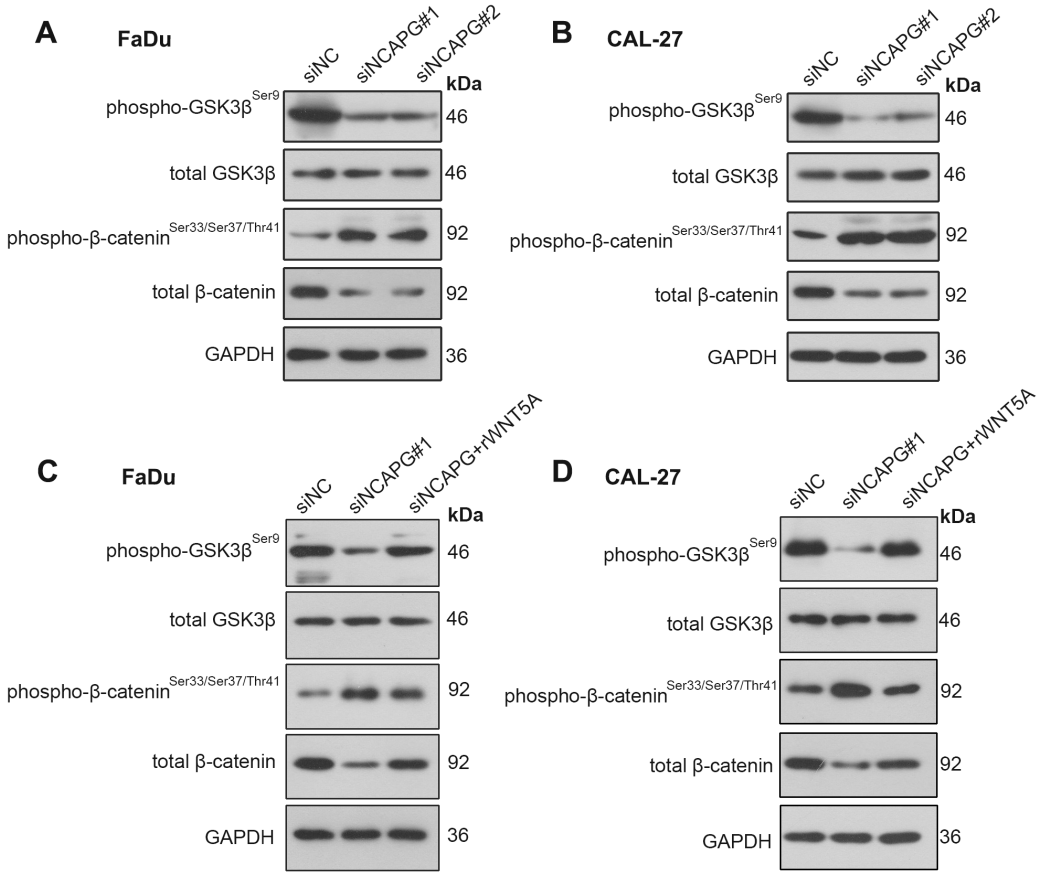

Figure 6. Knockdown of NCAPG suppressed the GSK-3β/β-catenin signaling in OSCC cells. Effects of NCAPG knockdown on the expression of the GSK-3β/β-Catenin signaling-related molecules in two OSCC cell lines FaDu (A) and CAL-27 (B), as detected by western blotting analysis. Recombinant WNT5A (rWNT5A) partially counteracted the effects of NCAPG knockdown on the expression of the GSK-3β/β-Catenin signaling-related molecules in FaDu (C) and CAL-27 (D) cells.

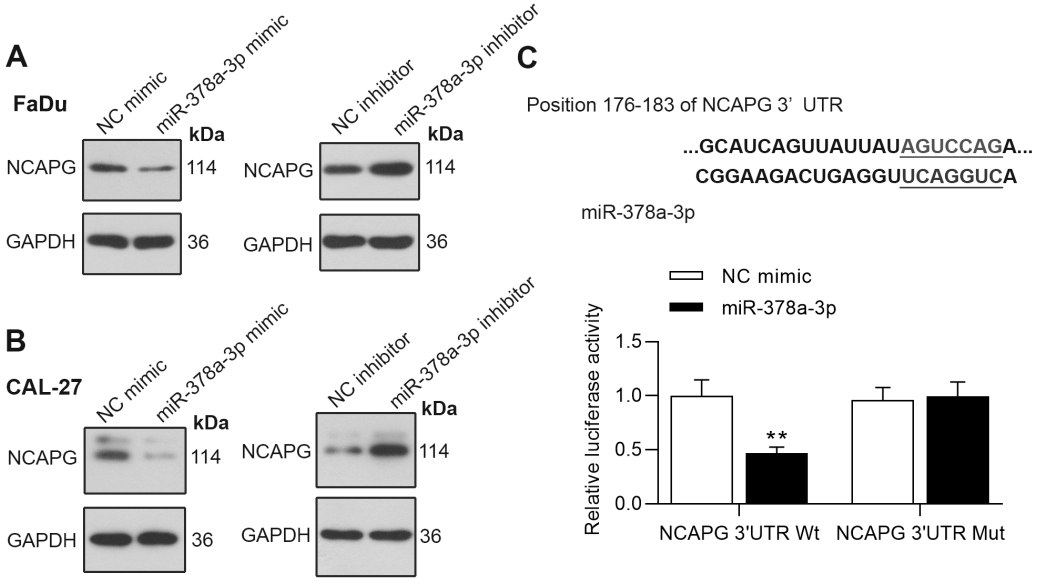

Figure 7. miR-378a-3p was a potential upstream regulator of NCAPG. Western blotting analysis of NCAPG expression in two OSCC cell lines FaDu (A) and CAL-27 (B) after transfection with miR-378a-3p mimic or inhibitor. C) Effect of miR-378a-3p on the luciferase activity of reporters containing wild type (wt) or mut (mutant type) NCAPG 3'-UTR sequences.

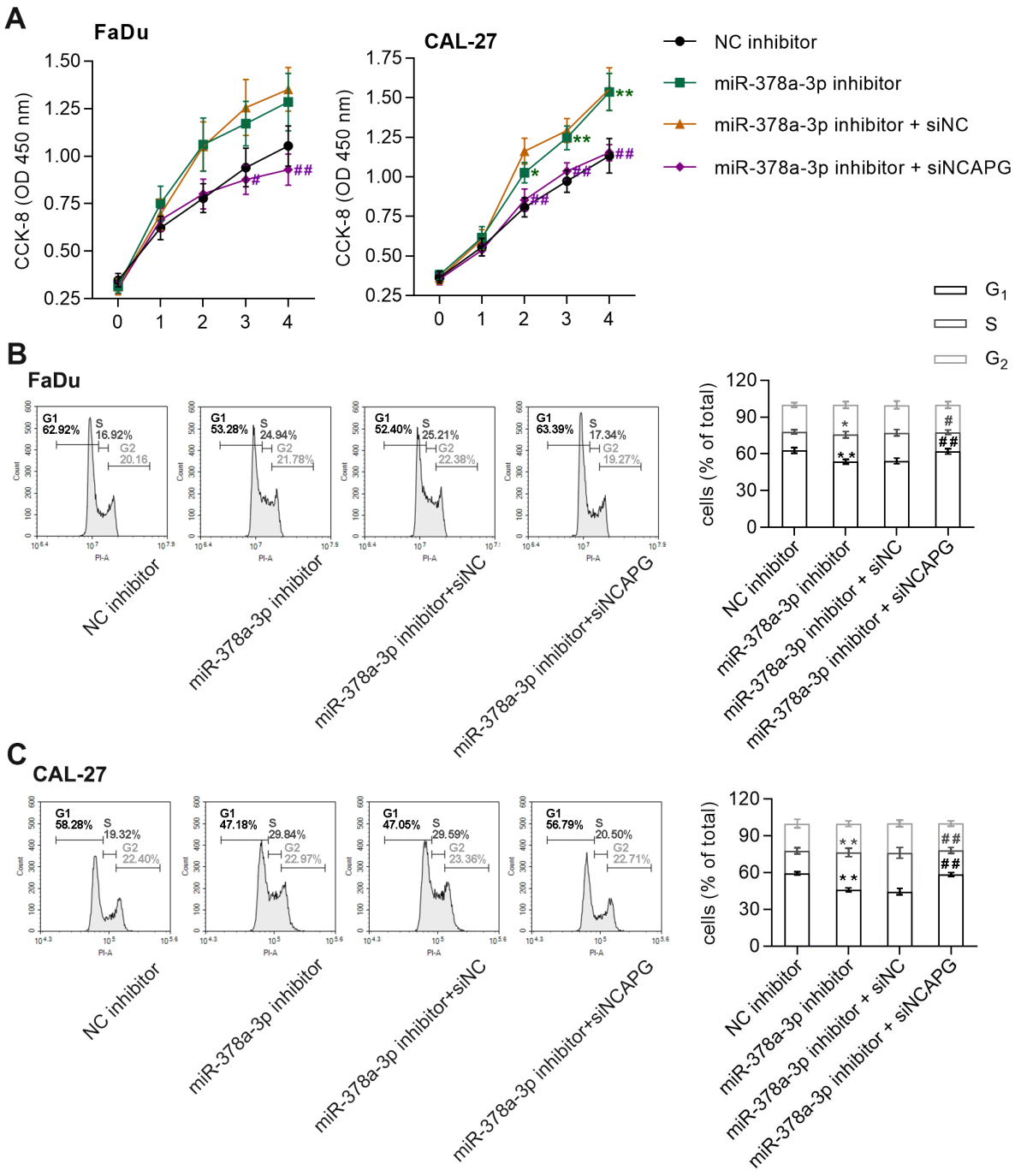

Figure 8. Knockdown of NCAPG counteracted the effects of miR-378a-3p on OSCC cell proliferation and cell cycle arrest. A) NCAPG silencing counteracted the effects of miR-378a-3p inhibitors on proliferation of FaDu and CAL-27 cells. NCAPG silencing counteracted the effects of miR-378a-3p inhibitors on cell cycle of FaDu (B) and CAL-27 (C) cells. P values are calculated by a two-tailed, unpaired t-test. *p<0.05; **p<0.01; compared with the NC inhibitor group; #p<0.05; ##p<0.01; compared with the miR-378a-3p inhibitor+siNC group.

across diverse types of cancer [9–11, 14]. However, its role in OSCC is still elusive. Herein, we silenced the NCAPG expression in two OSCC cell lines using siRNAs to perform loss-of-function studies and further explored its effect on OSCC cell proliferation, cell cycle progression, and apoptosis. We observed that the knockdown of NCAPG significantly inhibited OSCC cell proliferative ability, induced cell cycle arrest at the G1 phase, and inhibited apoptosis by performing

functional investigations. Simultaneously, we observed that there were obvious changes in the expression of related molecules including PCNA, Cyclin D1, CDK1, caspase-3, and caspase-9, further demonstrating the regulatory role of NCAPG in OSCC progression.

The pathogenesis of OSCC involves multiple signaling pathways, among which the Wnt/β-catenin pathway is intensely implicated [20–22]. The canonical Wnt/β-catenin pathway participates in multiple cellular processes, enabling tumor cells to proliferate, prolong survival, and gain invasiveness [23]. Glycogen synthase kinase 3β (GSK3β) is an evolutionarily conserved serine/threonine protein kinase that functions in cell proliferation, signaling, and metabolic pathways during cancer progression [24]. Notably, GSK3β is a key participant in the transduction of the canonical Wnt pathway [25]. It is generally accepted that reduction of the GSK3β ser9-phosphorylation leads to the increase of GSK-3β activity, followed by the promotion of β-catenin phosphorylation [26]. Phosphorylation of β-catenin at N-terminal Ser and Thr residues by GSK-3β results in its ubiquitylation and proteasomes-mediated degradation in the cytosol [27], thereby leading to an inhibition of carcinogenesis and tumor progression [28]. Consistently in this study, we discovered that inhibition of NCAPG reduced the ser9-phosphorylation of GSK3β, and increased the phosphorylation of β-catenin in OSCC cells. Moreover, we observed that the addition of rWNT5A abolished the inhibitory effect of NCAPG silencing on the GSK-3β/β-catenin signaling. These findings indicated that NCAPG knockdown suppressed the Wnt/β-catenin signaling pathway by promoting the degradation of β-catenin, thereby affecting OSCC progression.

Intriguingly, our lab previously reported that miR-378-3p/5p represses the proliferation and metastasis of oral squamous carcinoma cells by inhibiting KLK4 [29, 30]. By using online prediction software, we found that NCAPG may also be a target gene of miR-378a-3p (previously miR-378). So, we performed a dual-luciferase reporter assay to verify this hypothesis. We observed that not only NCAPG 3'UTR bound with hsa-miR-378a-3p, but also the NCAPG depletion counteracted the effect of the miR-378a-3p inhibitor on OSCC cell proliferation and cell cycle progression, suggesting that NCAPG serves as a downstream mechanism on miR-378a-3p regulating OSCC progression.

Taken together, we gather evidence that NCAPG, mediated by miR-378a-3p, functions as a tumor promoter in OSCC via regulation of the GSK-3β/β-catenin signaling in this work. Our findings demonstrated that NCAPG may serve as a promising new target in OSCC therapy.

References

[1] POLVERINI PJ, D'SILVA NJ, LEI YL. Precision Therapy of Head and Neck Squamous Cell Carcinoma. J Dent Res 2018; 97: 614–621. https://doi.org/10.1177/0022034518769645

[2] CHAI AWY, LIM KP, CHEONG SC. Translational genomics and recent advances in oral squamous cell carcinoma. Semin Cancer Biol 2020; 61: 71–83. https://doi.org/10.1016/j.semcancer.2019.09.011

[3] CHI AC, DAY TA, NEVILLE BW. Oral cavity and oropharyngeal squamous cell carcinoma--an update. CA Cancer J Clin 2015; 65: 401–421. https://doi.org/10.3322/caac.21293

[4] CHATTOPADHYAY I, VERMA M, PANDA M. Role of Oral Microbiome Signatures in Diagnosis and Prognosis of Oral Cancer. Technol Cancer Res Treat 2019; 18: 1533033819867354. https://doi.org/10.1177/1533033819867354

[5] MATSUURA D, VALIM TD, KULCSAR MAV, PINTO FR, BRANDÃO LG et al. Risk factors for salvage surgery failure in oral cavity squamous cell carcinoma. Laryngoscope 2018; 128: 1113–1119. https://doi.org/10.1002/lary.26935

[6] JÄGER D, STOCKERT E, JÄGER E, GÜRE AO, SCANLAN MJ et al. Serological cloning of a melanocyte rab guanosine 5'-triphosphate-binding protein and a chromosome condensation protein from a melanoma complementary DNA library. Cancer Res 2000; 60: 3584–3591

[7] EBERLEIN A, TAKASUGA A, SETOGUCHI K, PFUHL R, FLISIKOWSKI K et al. Dissection of genetic factors modulating fetal growth in cattle indicates a substantial role of the non-SMC condensin I complex, subunit G (NCAPG) gene. Genetics 2009; 183: 951–964. https://doi.org/10.1534/genetics.109.106476

[8] MURPHY LA, SARGE KD. Phosphorylation of CAP-G is required for its chromosomal DNA localization during mitosis. Biochem Biophys Res Commun 2008; 377: 1007–1011. https://doi.org/10.1016/j.bbrc.2008.10.114

[9] GONG C, AI J, FAN Y, GAO J, LIU W et al. NCAPG Promotes The Proliferation Of Hepatocellular Carcinoma Through PI3K/AKT Signaling. Onco Targets Ther 2019; 12: 8537–8552. https://doi.org/10.2147/ott.s217916

[10] ZHANG Q, SU R, SHAN C, GAO C, WU P. Non-SMC Condensin I Complex, Subunit G (NCAPG) is a Novel Mitotic Gene Required for Hepatocellular Cancer Cell Proliferation and Migration. Oncol Res 2018; 26: 269–276. https://doi.org/10.3727/096504017x15075967560980

[11] ZHANG X, WANG H, HAN Y, ZHU M, SONG Z et al. NCAPG Induces Cell Proliferation in Cardia Adenocarcinoma via PI3K/AKT Signaling Pathway. Onco Targets Ther 2020; 13: 11315–11326. https://doi.org/10.2147/ott.s276868.

[12] ZHANG X, ZHU M, WANG H, SONG Z, ZHAN D, et al. Overexpression of NCAPG inhibits cardia adenocarcinoma apoptosis and promotes epithelial-mesenchymal transition through the Wnt/β-catenin signaling pathway. Gene 2021; 766: 145163. https://doi.org/10.1016/j.gene.2020.145163

[13] ARAI T, OKATO A, YAMADA Y, SUGAWARA S, KUROZUMI A et al. Regulation of NCAPG by miR-99a-3p (passenger strand) inhibits cancer cell aggressiveness and is involved in CRPC. Cancer Med 2018; 7: 1988–2002. https://doi.org/10.1002/cam4.1455

[14] JIANG L, REN L, CHEN H, PAN J, ZHANG Z et al. NCAPG confers trastuzumab resistance via activating SRC/STAT3 signaling pathway in HER2-positive breast cancer. Cell Death Dis 2020; 11: 547. https://doi.org/10.1038/s41419-020-02753-x

[15] LEEMANS CR, BRAAKHUIS BJ, BRAKENHOFF RH. The molecular biology of head and neck cancer. Nat Rev Cancer 2011; 11: 9–22. https://doi.org/10.1038/nrc2982

[16] KURIHARA-SHIMOMURA M, SASAHIRA T, NAKASHIMA C, KUNIYASU H, SHIMOMURA H et al. The Multifarious Functions of Pyruvate Kinase M2 in Oral Cancer Cells. Int J Mol Sci 2018; 19. https://doi.org/10.3390/ijms19102907

[17] LINDEMANN A, TAKAHASHI H, PATEL AA, OSMAN AA, MYERS JN. Targeting the DNA Damage Response in OSCC with TP53 Mutations. J Dent Res 2018; 97: 635–644. https://doi.org/10.1177/0022034518759068

[18] GAN CP, SAM KK, YEE PS, ZAINAL NS, LEE BKB et al. IFITM3 knockdown reduces the expression of CCND1 and CDK4 and suppresses the growth of oral squamous cell carcinoma cells. Cell Oncol (Dordr) 2019; 42: 477–490. https://doi.org/10.1007/s13402-019-00437-z

[19] KIMURA K, CUVIER O, HIRANO T. Chromosome condensation by a human condensin complex in Xenopus egg extracts. J Biol Chem 2001; 276: 5417–5420. https://doi.org/10.1074/jbc.C000873200

[20] YANG YT, WANG YF, LAI JY, SHEN SY, WANG F et al. Long non-coding RNA UCA1 contributes to the progression of oral squamous cell carcinoma by regulating the WNT/β-catenin signaling pathway. Cancer Sci 2016; 107: 1581–1589. https://doi.org/10.1111/cas.13058

[21] LIU L, JIANG H, ZHAO J, WEN H. MiRNA-16 inhibited oral squamous carcinoma tumor growth in vitro and in vivo via suppressing Wnt/β-catenin signaling pathway. Onco Targets Ther 2018; 11: 5111–5119. https://doi.org/10.2147/ott.s153888

[22] SHAO TR, ZHENG ZN, CHEN YC, WU QQ, HUANG GZ et al. LncRNA AC007271.3 promotes cell proliferation, invasion, migration and inhibits cell apoptosis of OSCC via the Wnt/β-catenin signaling pathway. Life Sci 2019; 239: 117087. https://doi.org/10.1016/j.lfs.2019.117087

[23] XIE J, HUANG L, LU YG, ZHENG DL. Roles of the Wnt Signaling Pathway in Head and Neck Squamous Cell Carcinoma. Front Mol Biosci 2020; 7: 590912. https://doi.org/10.3389/fmolb.2020.590912

[24] LIN J, SONG T, LI C, MAO W. GSK-3β in DNA repair, apoptosis, and resistance of chemotherapy, radiotherapy of cancer. Biochim Biophys Acta Mol Cell Res 2020; 1867: 118659. https://doi.org/10.1016/j.bbamcr.2020.118659

[25] HADI F, AKRAMI H, SHAHPASAND K, FATTAHI MR. Wnt signalling pathway and tau phosphorylation: A comprehensive study on known connections. Cell Biochem Funct 2020; 38: 686–694. https://doi.org/10.1002/cbf.3530

[26] YAN DY, TANG J, CHEN L, WANG B, WENG S et al. Imperatorin promotes osteogenesis and suppresses osteoclast by activating AKT/GSK3 β/β-catenin pathways. J Cell Mol Med 2020; 24: 2330–2341. https://doi.org/10.1111/jcmm.14915

[27] SCHAEFER KN, PEIFER M. Wnt/Beta-Catenin Signaling Regulation and a Role for Biomolecular Condensates. Dev Cell 2019; 48: 429–444. https://doi.org/10.1016/j.devcel.2019.01.025

[28] STEWART DJ. Wnt signaling pathway in non-small cell lung cancer. J Natl Cancer Inst 2014; 106: djt356. https://doi.org/10.1093/jnci/djt356

[29] CUI Z, BAO X, LIU Q, LI Q, HUANG L et al. MicroRNA-378-3p/5p represses proliferation and induces apoptosis of oral squamous carcinoma cells via targeting KLK4. Clin Exp Pharmacol Physiol 2020; 47: 713–724. https://doi.org/10.1111/1440-1681.13235

[30] CUI Z, SUN S, LIU Q, ZHOU X, GAO S et al. MicroRNA-378-3p/5p suppresses the migration and invasiveness of oral squamous carcinoma cells by inhibiting KLK4 expression. Biochem Cell Biol 2020; 98: 154–163. https://doi.org/10.1139/bcb-2019-0017